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Platelet proteomics and its advanced application for research of blood stasis syndrome and activated blood circulation herbs of Chinese medicine

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The development of novel and efficient antiplatelet agents that have few adverse effects and methods that improve antiplatelet resistance has long been the focus of international research on the prevention and treatment of cardiovascular and cerebrovascular diseases. Recent advances in platelet proteomics have provided a technology platform for high-quality research of platelet pathophysiology and the development of new antiplatelet drugs. The study of blood stasis syndrome (BSS) and activated blood circulation of traditional Chinese medicine (TCM) is one of the most active fields where the integration of TCM and western medicine in China has been successful. Activated blood circulation herbs (ABC herbs) of Chinese medicine are often used in the treatment of BSS. Most ABC herbs have antiplatelet and anti-atherosclerosis activity, but knowledge about their targets is lacking. Coronary heart disease (CHD), BSS, and platelet activation are closely related. By screening and identifying activated platelet proteins that are differentially expressed in BSS of CHD, platelet proteomics has helped researchers interpret the antiplatelet mechanism of action of ABC herbs and provided many potential biomarkers for BSS that could be used to evaluate the clinical curative effect of new antiplatelet drugs. In this article the progress of platelet proteomics and its advanced application for research of BSS and ABC herbs of Chinese medicine are reviewed.

platelet proteomics, blood stasis syndrome, activated blood circulation, coronary heart disease, Chinese medicine, activated blood circulation herbs

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Platelets are micro bioactive matter, which originates from the cytoplasm of bone marrow megakaryocyte. They play a pivotal role in many pathophysiologic processes, including hemostasis, inflammation, thrombogenesis, and organ-graft rejection. Acute thrombus formation, secondary to rupture of vulnerable atherosclerotic plaque triggered by platelet activation and inflammation, is the principal pathophysiological basis of acute vascular events. Antiplatelet drugs such as aspirin and clopidogrel are used in the prevention and treatment of cardiovascular and cerebrovascular diseases [1]. However, prolonged treatment with dual or triple antiplatelet drugs revealed a diversity of platelet reactions, including either poor or hyper responses. In poor responses, recurrent cardiovascular events still occurred in individuals who were taking enough antiplatelet drugs in a timely manner, which led to antiplatelet resistance [2,3]. The hyper response resulted in increased risk of bleeding in the digestive and nervous systems, which limits the clinical administration of antiplatelet drugs. These findings suggested that other pathways capable of stimulating platelet activation may exist. The discovery of novel classes of antiplatelet

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agents that possess high efficiency with few adverse effects and the development of methods to improve antiplatelet resistance have long been research focuses for the prevention and treatment of cardiovascular and cerebrovascular diseases.

1 Platelet proteomics

Proteomics focuses on the composition and changing profiles of all the proteins in a cell or tissue, which provides new ideas and strategies for disclosing the molecular mechanisms behind serious diseases and identifying targets for intervention drugs. Platelets are anuclear cells, which contain intracellular granules, such as alpha granules, dense body, and lysosomes. Platelets are ideal targets for proteomics because of their functions in protein synthesis and modification followed by the transcription of megakaryocyte. Proteomics was used to identify differential expression patterns between resting and activated platelets proteins under resting or activating condition, and signal transduction pathways related to platelet metabolism were also identified [4]. Thus, platelet functional changes in normal and pathological states were identified, which enriched the understanding of the biological function of platelets. High-throughput proteomics has made possible the screening of new antiplatelet agents. Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) is an ideal technique for the analysis of differentially expressed proteins. 2-D DIGE has wonderful reproducibility, which helps avoid the low reproducibility and serious systematic variations that often occur in traditional two-dimensional gel electrophoresis. Matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF-TOF) tandem mass spectrometry accompanied by 2-D DIGE, has high sensitivity and specificity. Platelet proteomics has been applied successfully to early diagnosis and new drug research, and to understand the development of serious diseases, such as hematological diseases and coronary heart disease. The research model usually contains three steps: screening, identification (qualitative and quantitative), and functional analysis [5–7].

In a 2012 paper, Burkhart et al. [8] reported the first comprehensive and quantitative human platelet proteome by quantitative mass spectrometry. They identified more than 2500 phosphorylation sites, almost 4000 unique proteins, and estimated the copy numbers per platelet for 3700 of them. In addition, 1900 proteins were quantified between four different donors (intersubject variation) and 1500 proteins between three different blood samples from the same volunteer (intrasubject variation). The results demonstrated that, in both cases, 85% of the platelet proteome showed no variation. This study provided a basis for subsequent functional analyses of screened platelet proteins.

2 Platelet proteomics and blood stasis syndrome of Chinese medicine

Proteomics looks at the characteristics of protein activity on the whole, which is similar to the holistic view in traditional Chinese medicine (TCM) theory. In recent years, clinical medicine has moved from a fragmented approach to a more holistic integrative model [9]. In the real medical environment, western medicine and TCM have merged to become the Chinese integrative medicine, a unique medical system in China. A research model that combines “disease”, diagnosed by western medicine, and “syndrome”, recognized by TCM theory, is one of the directions in which Chinese integrative medicine will develop in the future [10]. Functional proteomics could be used to detect the differential expression patterns of proteins in typical syndromes defined by TCM theory. All the proteins associated with a syndrome could be analyzed and characterized to describe the TCM syndrome at the overall protein expression level and to provide scientific evidence for the syndrome [11].

Blood stasis syndrome (BSS) is defined by TCM theory and has the status of platelet activation. A previous study demonstrated that coronary heart disease (CHD), BSS, and platelet activation were closely related [12]. Using the well-established diagnostic criteria of BSS, we set up a diagnostic criteria of BSS of CHD [13]. Platelets play an important role in the formation of coronary artery thrombosis, and activated platelets not only participate in the formation of blood clots, but also release a variety of vascular active substances, cytokines and growth factors that are involved in the formation and development of atherosclerosis. BSS has a close relationship with many pathological physiology changes, including microcirculation disturbance, high blood viscous state, and platelet activation and adhesion. BSS is the most common TCM syndrome type of CHD, and a previous study reported that BSS of CHD had the status of platelet activation [14].

Taking the BSS of CHD and activated blood circulation (ABC) herbs as the starting point, we applied platelet proteomics to study BSS and the effects of ABC herbs. In a previous study [15], 45 differentially expressed platelet proteins between BSS of CHD and non-BSS of CHD (Figure 1) were identified by platelet proteomics and screened based on a three-dimensional diagram, isoelectric point, molecular weight, and degree of information matching to the real position in the gel. As a result, 23 platelet protein points were identified. Mass spectrum successfully identified 14 different platelet proteins (Table 1), and after removing four redundancies, 10 differentially expressed platelet proteins with reliable supporting data remained. Most of these proteins were annotated as platelet cytoskeleton proteins or platelet membrane proteins. In addition to causing changes in the membrane proteins, platelet activation, which depends on the regulation of platelet cytoskelet-

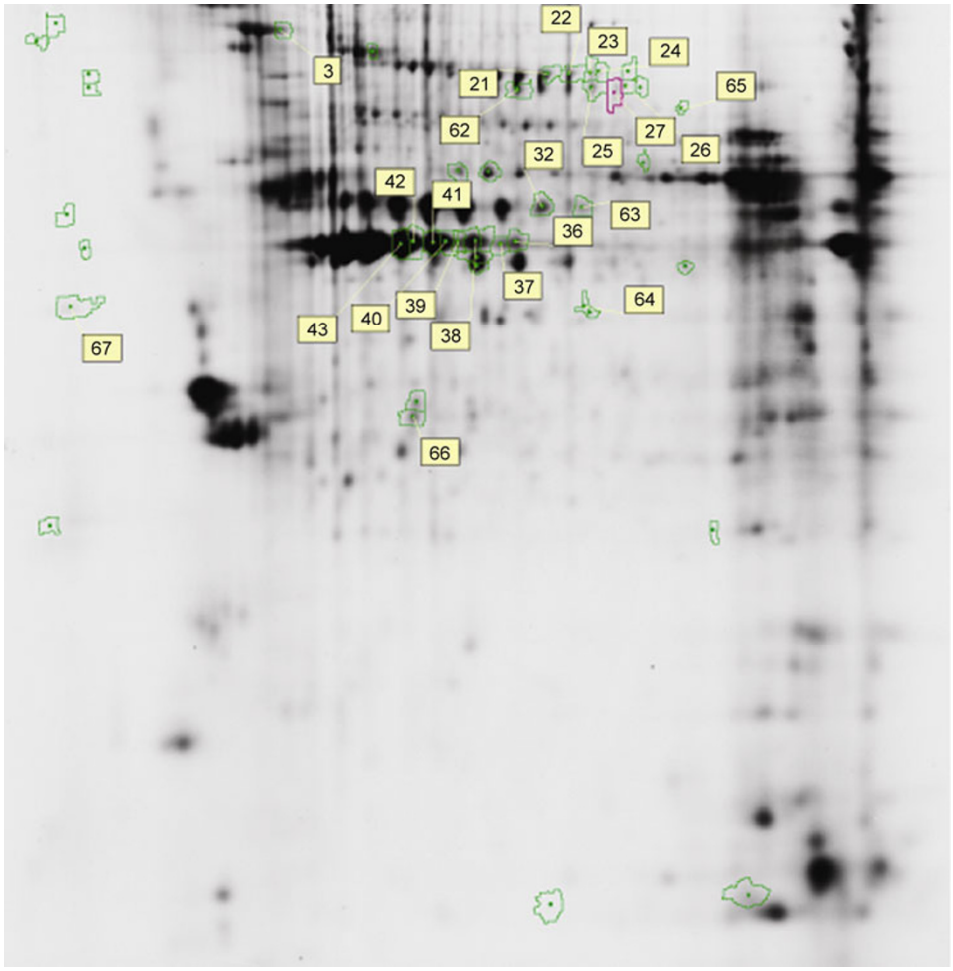

Figure 1 Differentially expressed platelet proteins between BSS and non-BSS of CHD groups (reproduced from Figure 31 of reference [15] with the author's authorization).

al protein, can also trigger a series of morphological changes from inviscid discotic circulating platelets into a pastelike protruding platelet jelly. For clinical verification, we chose gelsolin because a literature search revealed that it has a clear function. Our results confirmed that the platelet gelsolin level in BBS patients of CHD increased significantly compared with that in non-BSS patients of CHD and healthy subjects [16], revealing that gelsolin had a close relationship with BSS of CHD and indicating that platelet cytoskeletal protein may play an important role in the development of BSS of CHD. In addition, we observed a clinical correlation between the platelet gelsolin level of different types of CHD (stable CHD and acute coronary syndrome), showing that the platelet gelsolin level had a positive relation with acute coronary syndrome [17].

The role of gelsolin in cardiovascular diseases is attracting more and more attention worldwide and several studies have indicated that gelsolin plays an important role in the development of CHD, arrhythmia, and ventricular remodeling after acute myocardial infarction [18,19]. Gelsolin is an important cytoskeletal protein and a high concentration of calcium activates the secretion of gelsolin. After infarction, myocardial F-actin is released into the circulation, which can be fatal for endothelial cells and micrangium. An extracellular actin scavenger system is therefore likely to exist [20], and plasma gelsolin and Gc-globulin, another extracellular actin-binding protein, have been regarded as potentially important components of a system that would be capable of removing F-actin from the circulation and inhibiting F-actin elongation. Platelet gelsolin and the platelet activation level of BSS of CHD have a high positive correlation. During platelet activation of BSS of CHD, the composition of cytoskeletal protein varies, including the platelet gelsolin and F-actin concentrations. We speculate that there might be an abnormal increase in platelet gelsolin as a result of reactive restructuring of platelet cytoskeleton because of the depletion of plasma gelsolin or increased calcium influx in platelets during the development of BSS of CHD. This process could promote the platelet activation and transformation involved in the progress of BSS of CHD. Therefore, platelet gelsolin is a new potential biomarker of BSS in CHD [16].

Table 1 Identified platelet proteins differentially expressed between BSS and non-BSS of CHD groups[a)]

Accession No.	Protein name	Protein MW	Protein	Protein	Protein score C.I.%	Total ion score	Total ion C.I.%	Spot number
IPI00218628	Gene_Symbol=ITGA2B Isoform 2 of Integrin alpha-IIb	109518.5	5.17	751	100	651	100	3
IPI00877792	Gene_Symbol=FGG 50 kD protein	50290.4	5.71	141	100	62	99.929	32
IPI00739539	Gene_Symbol=A26C1B ANKRD26-like	121292.7	5.86	170	100	143	100	36
IPI00894365	Gene_Symbol=ACTB cDNA FLJ52842,	39200.5	5.4	321	100	252	100	37
IPI00021439	Gene_Symbol=ACTB Actin, cytoplasmic 1	41709.7	5.29	445	100	330	100	38
IPI00021439	Tax_Id=9606 Gene_Symbol=ACTB Actin, cytoplasmic 1	41709.7	5.29	342	100	274	100	39
IPI00021439	Gene_Symbol=ACTB Actin, cytoplasmic 1	41709.7	5.29	440	100	379	100	40
IPI00021440	Gene_Symbol=ACTG1 Actin, cytoplasmic 2	41765.8	5.31	602	100	480	100	41
IPI00021439	Gene_Symbol=ACTB Actin, cytoplasmic 1	41709.7	5.29	588	100	478	100	42
IPI00894365	Gene_Symbol=ACTB cDNA FLJ52842, highly similar to Actin, cytoplasmic 1	39200.5	5.4	442	100	326	100	43
IPI00794523	Tax_Id=9606 Gene_Symbol=ACTG1 cDNA FLJ43573 fis, clone RECTM2001691, highly similar to Actin, cytop	28193	5.2	236	100	189	100	66
IPI00298497	Gene_Symbol=FGB Fibrinogen beta chain	55892.3	8.54	80	99.924	59	99.867	63
IPI00009865	Gene_Symbol=KRT10 Keratin, type I cytoskeletal 10	59474.9	5.13	141	100	56	99.903	23
IPI00796316	Gene_Symbol=GSN cDNA FLJ53327, highly similar to Gelsolin	77741.1	5.47	83	99.958	40	95.153	62

a) Reproduced from Table 13 of reference [15] with the author's authorization. Accession No. in italics and underlined indicate the redundant proteins. Spot number indicates the number of differentially expressed platelet proteins in protein glue figure.

3 Platelet proteomics and research of activated blood circulation herbs of Chinese medicine

ABC herbs and formulas are the main drugs that are used in the clinical treatment of BSS in TCM theory. During the past 50 years, much theoretical and experimental progress has been made in the fields of TCM. As a result, the regulation and principles of treatments that use ABC herbs and formulas have been clarified and such treatments are now accepted by the medical community all over the world [21]. Chinese and western medicines agree that stabling plaque and activating blood circulation can help prevent atherosclerosis and vulnerable plaque. In this context, the application of ABC herbs and formulas has valuable significance in reducing the risk of cardiovascular event [22]. Studies have shown that ABC herbs such as Danshen (*Salvia miltiorrhiza*), Danpi (*Paeonia suffruticosa* Andr.), Danggui (*Angelica sinensis*), Chishao (*Paeonia veitchii*), Puhuang (*Typha angustifolia pollen*), Chuanxiong (*Ligusticum sinense*) and Sanqi (*Panax notoginseng*), and formulas such as Xue-Fu-Zhu-Yu decoction, compositus Guan-Xin No.2, and Tao-Hong-Si-Wu decoction have antiplatelet and anti-inflammatory action. The effective constituents of ABC herbs include flavonoids (puerarin and carthamin yellow), alkaloids (ligustrazine and all the Chuanxiong alkaloids, terpenes (tanshinone and *Panax notoginseng* saponins), organic acids (tanshinol and salvianolic acid). Most of the previous research focused on pharmacology observations such as platelet aggregation rate and platelet receptors, and rarely on protein expression after platelet activation [23]. Therefore, studies on the targets and signaling pathways that are involved in reducing the risk of cardiovascular disease are needed. Platelet proteomics can help identify more potential targets, which may elucidate the mechanisms of action of the ABC herbs and formulas based on the TCM theory of "correspondence of formula to syndrome" [24].

3.1 Chishao and Chuanxiong

Chishao and Chuanxiong are classical ABC herbs that have been used for thousands of years in the prevention and treatment of cardiovascular diseases in China. Studies indicated that these two herbs have ideal antiplatelet aggregation and anti-thrombosis action. The Xiongshao capsule (XSC) is a patented drug developed from Xue-Fu-Zhu-Yu decoction. It is a classic formula that has been used for hundreds of years in China to activate blood circulation. The effective components of XSC are Chuanxiong rhizome and red peony (*Paeonia lactiflora*) root. Experimental studies have shown that XSC dilate coronary arteries, thereby improving myocardial ischemia and hypoxia, promoting antiplatelet aggregation, inhibiting smooth muscle proliferation, inhibiting lipid peroxidation, and promoting angiogen-

esis [25–27]. Clinical studies have shown that XSC can effectively prevent restenosis after percutaneous coronary intervention [28,29]. Platelet plays vital roles in the development of atherosclerosis, rupture of vulnerable plaque, and formation of thrombogenesis. By elucidating the antiplatelet mechanism and targets of XSC, new ideas for antiplatelet therapy may be revealed.

As was mentioned above, it was suggested that excessive F-actin at the platelet activation stage of BSS of CHD might deplete plasma gelsolin and directly stimulate platelet to secrete abnormal amounts of gelsolin. If this is the case, then platelet gelsolin may be the therapeutic target of the antiplatelet activation effect of ABC herbs and formulas. To further investigate these proposals, we build an experimental *in vitro* model system of platelet activation and an *in vivo* myocardial infarction rat model. We found that F-actin can induce platelet aggregation and activation *in vitro*, and a high concentration of F-actin can increase the platelet gelsolin level of activated platelet, which is similar to the effect of arachidonic acid reported previously [30]. Paeoniflorin and ligustrazine phosphate can inhibit platelet aggregation and activation *in vitro* and can also reduce the platelet gelsolin level of activated platelet. Aspirin had no such effect *in vitro*. The active ingredients of Chuanxiong rhizome and red peony root (chuangxiongol and paeoniflorin) can reduce the platelet gelsolin level, enhance the activity of the extracellular actin scavenger system, and inhibit platelet activation, which is similar to the effect of calcium antagonist [31]. The above series of studies confirmed the idea that platelet gelsolin may be an effective target for ABC herbs and formulas.

3.2 Sanqi and Danshen

Sanqi is a medicinal drug with a history of more than 500 years (according to the Ben Cao Gang Mu (Compendium of Materia Medica)), which contains saponin, flavones, and protein and non-protein amino acids. Saponin is found in higher proportions than the other components, and is the component that activates blood circulation. To identify the possible target proteins of Sanqi in platelets, comparative proteomics using two-dimensional gel electrophoresis was performed and the proteins that showed altered expression levels after Sanqi treatment were identified by MALDI-TOF tandem mass spectrometry. It was reported that Sanqi treatment regulated the levels of 12 proteins that play important roles in platelet activation, oxidative stress, and cytoskeleton. In the Sanqi-treated platelets compared with non-treated platelets, there were increased levels of growth factor receptor-bound protein 2 (Grb2), thrombospondin 1, and tubulin alpha 6, and decreased levels of thioredoxin, Cu-Zn superoxide dismutase, DJ-1 protein, peroxiredoxin 3, thioredoxin-like protein 2, ribonuclease inhibitor, potassium channel subfamily V member 2, myosin regulatory light chain 9, and laminin receptor [32].

Danshen was first recorded in Sheng Nong's Herbal Classic work as having the effect of activating blood circulation and it is still widely used in TCM for the treatment of cardiovascular disorders. The chemical components of Danshen are fat-soluble diterpene quinines (tanshinone I, IIA, IIB) and water-soluble phenolic acids (salvianolic acid B and tanshinol). Salvianolic acid B has been shown to be an active component. Differential proteomic analysis by two-dimensional electrophoresis was conducted to check the protein expression profiles of rat platelets with and without salvianolic acid B treatment. Proteins with altered expression levels after salvianolic acid B exposure were identified by MALDI-TOF tandem mass spectrometry. Salvianolic acid B treatment was found to regulate the expression of 20 proteins such as platelet aggregation and blood coagulation related proteins (e.g., the platelet-activating factors acetylhydrolase Ib-β and prothrombinase FGL-2), cellular transmembrane signal transduction related proteins (e.g., potassium voltage-gated channel, 14-3-3ε and copine J), material energy metabolism related proteins (e.g., aldolase A), cytoskeletal protein (coronins and tropomyosin), and other proteins such as heat shock-related 70, peroxiredoxin-2, and zinc finger proteins [33].

4 Conclusion and perspectives

Chinese integrative medicine is an important part of complementary and alternative medicine in the world. Its outstanding achievements include arsenic trioxide for leukemia, artemisinin and its derivative for malaria, and activated blood circulation herbs for cardiovascular diseases, all of which have been approved worldwide in the medical field [34]. The future development of TCM needs the inputs that modern science can provide [35]. An exploration of the mechanisms of action of the existing herbs and formulas is one of the major directions that Chinese integrative medicine can take.

ZHENG is a syndrome or pattern that is at the core of TCM theory. The essence of ZHENG is not only the demand for the development of TCM, but is also the inevitable choice of the modernization of TCM. Platelet plays an important role in the formation of atherosclerotic thrombosis, which is the common pathology that underlies many cardiovascular and cerebrovascular diseases. The emergence and development of platelet proteomics has provided a technological platform that can be used to explore the essence of ZHENG based on the "combination of disease and syndrome". Platelet proteomics could be applied to identify potential biomarkers of ZHENG by comparing platelet proteomes from patients with different ZHENG syndromes. Guided by the TCM theory of "correspondence of formula to syndrome" or "correlation between formula and syndrome" [36], targets could be identified by analyzing the expression levels of platelet proteins before and after inter-

vention with a therapy or formula, the mechanism of action of formulas could be obtained, and the clinical effects of new antiplatelet drugs could be screened and evaluated. Currently, platelet proteomics tends to stop at the screening and identification stage, and only rarely is extended to the functional analysis of the differentially expressed platelet proteins. The difficulty of identifying low abundance platelet proteins and methods to analyze the massive amounts of data that are obtained, are issues that need to be addressed in future platelet proteomics research. The abundant research achievements that have already been obtained using platelet proteomic techniques and its continued application to the study of BSS and ABC herbs, will surely reveal many more amazing new results and findings, which in turn will help decrease the morbidity and mortality caused by cardiovascular and cerebrovascular diseases.

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1 Michelson A D. Antiplatelet therapies for the treatment of cardiovascular disease. Nat Rev Drug Discov, 2010, 9: 154–169
2 Gergely F, Andrea F, Gabriella P, et al. Clinical importance of aspirin and clopidogrel resistance. World J Cardiol, 2010, 2: 171–186
3 Pena A, Collet J P, Hulot J S. Can we override clopidogrel resistance? Circulation, 2009, 119: 2854–2857
4 Yang Y L, Liu W J. Research progress of platelet proteomics. J Exp Hematol, 2012, 20: 777–781
5 Di Michele M, Van Geet C, Freson K. Proteomics to unravel platelet-related diseases and identify novel anti-platelet drugs. Curr Med Chem, 2012, 19: 4662–4670
6 van der Meijden P E, Heemskerk J W. Platelet protein shake as playmaker. Blood, 2012, 120: 2931–2932
7 Di Michele M, Van Geet C, Freson K. Recent advances in platelet proteomics. Expert Rev Proteomics, 2012, 9: 451–466
8 Burkhart J M, Vaudel M, Gambaryan S, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood, 2012, 120: e73–82
9 Chen K J, Liu Y. Clinical medicine: A holistic integrative medicine in Nature. Med Philos, 2013, 3A: 12–13
10 Chen K J. The Treatment viewpoints and clinical practice of disease identification and syndrome typing (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2011, 31: 1016–1017
11 Zhou H G, Chen H B, Zhou X P, et al. Proteomics is the important technology platform for Chinese medicine pathogenesis research (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2012, 32: 990–993
12 Chen K J, Xue M, Yin H J. The relationship between platelet activation and coronary heart disease and blood stasis syndrome (in Chinese). Shoudu Yi Ke Da Xue Xue Bao, 2008, 29: 266–269
13 Fu C G, Gao Z Y, Wang P L, et al. Study on the diagnostic criteria for coronary heart disease patients of blood stasis syndrome (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2012, 32: 1285–1286
14 Xue M, Chen K J, Yin H J. Relationship between platelet activation related factors and polymorphism of related genes in patients with coronary heart disease of blood-stasis syndrome. Chin J Integr Med, 2008, 14: 267–273
15 Li X F. Study on platelet differential functional proteins of blood stasis syndrome in coronary heart disease and drug intervention. Dissertation for Doctoral Degree. Beijing: Xiyuan Hospital, China Academy of Chinese Medical Sciences, 2010
16 Liu Y, Yin H J, Chen K J. Research on the correlation between platelet gelsolin and blood-stasis syndrome of coronary heart disease. Chin J Integr Med, 2011, 17: 587–592
17 Liu Y, Yin H J, Jiang Y R, et al. Correlation between platelet gelsolin level and different types of coronary heart disease. Chin Sci Bull, 2012, 57: 631–638
18 Li G H, Shi Y, Chen Y, et al. Gelsolin regulates cardiac remodeling after myocardial infarction through DNase I-mediated apoptosis. Circ Res, 2009, 104: 896–904
19 Liu Y, Jiang Y R, Yin H J, et al. Gelsolin and cardiovascular diseases. Mol Cardiol China, 2011, 11: 50–53
20 Lee W M, Galbraith R M. The extracellular actin scavenger system and actin toxicity. N Engl J Med, 1992, 326: 1335–1341
21 Chen K J, Li L D, Weng W L. Blood stasis and research of activating blood circulation and eliminating stasis (in Chinese). Zhong Xi Yi Jie He Xin Nao Xue Guan Bing Za Zhi, 2005, 1: 1–2
22 Chen K J. Explore the possibilities of Chinese herb and formulas for promoting blood circulation and removing blood stasis on reducing the cardiovascular risk (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2008, 5: 389
23 Liu Y, Yin H J, Shi D Z, et al. Chinese herb and formulas of promoting blood circulation and removing blood stasis and antiplatelet therapies. Evid-based Compl Alt Med, 2012, 184503, doi: 10.1155/2012/184503
24 Lu J, Liu J, Shen X P. Application of proteomics in pharmacology of traditional Chinese medicine research. Pharmacol Clin Chin Mater Med,2012, 28: 243–247
25 Xu H, Wen C, Chen K J, et al. Study on the effect of rhizoma Chuanxiong, radix paeoniae rubra and the compound of their active ingredients, Xiongshao Capsule, on stability of atherosclerotic plaque in ApoE$^{-/-}$ mice (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2007, 27: 513–518
26 Li L Z, Liu J G, Ma L B, et al. Effect of Xiongshao Capsule on lipid metabolism and platelet aggregation in experimental atherosclerosis rabbits (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2008, 28: 1100–1103
27 Xu F Q, Xu H, Liu J G, et al. Effects of Xiongshao Capsule on the proliferation of vascular smooth muscle cells in rabbits with atherosclerosis (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2008, 28: 912–917
28 Lu X Y, Shi D Z, Xu H, et al. Clinical study on effect of Xiongshao Capsule on restenosis after percutaneous coronary intervention (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2006, 26: 13–17
29 Chen K J, Shi D Z, Xu H, et al. XS0601 reduces the incidence of restenosis: a prospective study of 335 patients undergoing percutaneous coronary intervention in China. Chin Med J (Engl), 2006, 119: 6–13
30 Liu Y. Study on the role of platelet cytoskeletal protein gelsolin in the development of blood stasis syndrome in coronary heart disease and intervention mechanism of effective components of Chuanxiong rhizome and red peony root. Dissertation for Doctoral Degree. Beijing: Xiyuan hospital, China Academy of Chinese Medical Sciences, 2012
31 Liu Y, Yin H J, Jiang Y R, et al. Correlation between platelet gelsolin and platelet activation level in acute myocardial infarction rats and intervention effect of effective components of Chuanxiong rhizome and red peony root. Evid Based Compl Alternat Med, 2013, 985746, doi: 10.1155/2013/985746
32 Yao Y, Wu W Y, Guan S H, et al. Proteomic analysis of differential protein expression in rat platelets treated with notoginsengnosides. Phytomedicine, 2008, 15: 800–807

33 Ma C, Yao Y, Yue Q X, et al. Differential proteomic analysis of platelets suggested possible signal cascades network in platelets treated with salvianolic acid B. PLoS ONE, 2011, 6: e14692
34 Xu H, Chen K J. Complementary and alternative medicine: Is it possible to be mainstream? Chin J Integr Med, 2012, 18: 403–404
35 Wang T. Chinese medicine needs the baptism of science (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi, 2012, 32: 1014–1022
36 Xie M. The logical proposition and significance of "correlation between formula and syndrome" of Chinese medicine. J Beijing Univ Chin Med, 2003, 26: 11–12